PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 39/00, 39/02, C07K 14/74, 14/195, 14/435, G01N 33/53 — A1

(11) International Publication Number: **WO 97/24140**

(43) International Publication Date: 10 July 1997 (10.07.97)

(21) International Application Number: PCT/US97/00117

(22) International Filing Date: 2 January 1997 (02.01.97)

(30) Priority Data:

08/581,917	2 January 1996 (02.01.96)	US
08/635,860	22 April 1996 (22.04.96)	US

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(71) Applicant: THE BOARD OF TRUSTEES OF LELAND STANFORD JUNIOR UNIVERSITY [US/US]; Suite 350, 900 Welch Road, Palo Alto, CA 94304 (US).

(72) Inventors: CLAYBERGER, Carol; 812 Mayfield Avenue, Stanford, CA 94305 (US). KRENSKY, Alan, M.; 812 Mayfield Avenue, Palo Alto, CA 94305 (US).

(74) Agents: MILLMAN, Robert, A. et al.; Morrison & Foerster L.L.P., 2000 Pennsylvania Avenue, N.W., Washington, DC 20006-1888 (US).

(54) Title: INTERACTION OF HLA PROTEINS WITH MEMBERS OF THE HSP70 FAMILY OF PROTEINS

(57) Abstract

Present invention based on the identification of the molecular interaction which forms the basis of the immunosuppressive activity of the HLA-B2702.75-84 peptide. Specifically the present invention discloses that the HLA-B2702.75-84 peptide binds to members of the HSP70 family of proteins. Based on this observation, present invention provides methods of identifying agents which can be used to modulate immune system activity.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia
AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

INTERACTION OF HLA PROTEINS WITH MEMBERS OF THE HSP70 FAMILY OF PROTEINS

Technical Field

The field of this invention is the modification of immune system activity.

Background

The immune system is the subject of ever increasing scientific scrutiny. Despite the enormous interest in the immune system and the continuously expanding number of investigators, both academic and industrial, a complete understanding of the system continues to remain elusive. One of the major breakthroughs was identification of the interaction between the T-cell receptor and a major histocompatibility complex antigen. The identification that both class I and class II major histocompatibility complex antigens have a cleft which binds a small peptide provided a significant key to understanding T-cell specificity and T-cell restriction.

The understanding that the MHC served macrophages and B-cells in their role as antigen presenting cells, where the peptide served to define which T-cells could bind to the MHC-peptide complex still did not explain many other aspects of the T-cell response. It appeared that the binding of the T-cell receptor to the antigen presenting cell was not enough by itself to result in T-cell activation. The process of anergy or tolerization could not be explained by an activation process solely involving the T-cell receptor and the MHC antigen. In addition, there was the distinction between helper cells ($CD4^+$) and suppresser/cytotoxic cells ($CD8^+$). Some mechanism was necessary to associate the helper cells with class II MHC and the suppresser/cytotoxic cells with class I MHC. It was subsequently found that CD4 and CD8 participated in the MHC TCR complex by binding a loop of the MHC, enhancing the stability of the complex. In addition, other interactions were uncovered, apparently not directly associated with the T-cell receptor/MHC complex, where CD28 and B7 were found to bind. Thus, there appear to be a number of different interactions involved with the association of

the antigen presenting cell and the T-cell, which could result in tolerization or activation of the T-cell.

Because of the crucial role that the T-cell plays at the center of a major component of the immune system, it remains of great importance to be able to understand how T-cells are selected, activated or tolerized. By understanding the role that various participants play in T-cell activation, there will be opportunities to regulate the immune system, either enhancing the immune response, where one is dealing with vaccines, pathogens, neoplasia, or the like, or diminishing the immune response, where one is dealing with autoimmunity or organ transplantation.

Relevant Literature

Wan *et al.*, *J. Immunol.*, 1986. 137:3671 -4 describes the Bw4/Bw6 epitopes. Clayberger, *et al.* (1987) *Nature* 330:763-765 describe that HLA A2 peptides can regulate cytolysis by human allogeneic T lymphocytes. WO93/17699 describes the activity of peptides from the HLA-A and -B α1 25 and α2 helices in modulating CTL activity. HLA-B2702.75-84 peptide is described.

The HLA-B2702.75-84 peptide has been shown to be effective in modulating immune system activity, particularly the activity of T-cell function, particularly activation and T-cell mediated responses. Prior to the present invention, the underlying molecular mechanism by which the HLA-B2702.75-84 peptide exerted immunomodulating activity was not understood. The present invention has advanced the state of the art by identifying the molecular interaction involved in HLA-B2702.75-84 activity. By identifying the pertinent molecular interaction, the present invention provides a basis for identifying new immunomodulating agents which are more effective or more selective than HLA-B2702.75-84 and methods for modulating immune system activity using such identified compounds.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the identification of one of the mechanism by which immune responses, particularly those involving T-cells, are potentiated. In the following Examples, data are provided showing that members of the HSP70 class of proteins interact with peptides corresponding to members of the HLA proteins to inhibit immune responsiveness mediated by T-cells, for example Cytotoxic T-cell mediated killing (CTL) and T-cell differentiation.

The present invention is further based on identifying how fragments of HLA peptides, particularly the HLA-B2702.75-84 peptide, interact with a member of the HLA70 family of proteins to modulate immune responses. In the Examples, the ability of HLA-B2702.75-84 to block immune functions was shown to be mediated by the interaction of HLA-B2702.75-84 with a member of the HSP70 family of proteins. This observation is important because the target of HLA-B2702.75-84 was previously unknown in the art. The identified HLA-B2702.75-84/HSP70 interaction of the present invention can be used as a basis for making and identifying agents which can modulate immune responses. Competitive assays using HSP70 and the HLA-B2702.75-84 peptide, or a HLA-B2702.75-84 or HSP70 equivalent, can be used to identify compounds which block the same interactions as that blocked by the HLA-B2702.75-84. Additionally, peptide and protein modeling techniques can be used to study the specific interactions of the HLA-B2702.75-84 peptide with HSP70 to rationally design or rationally select agents for testing. Such agents can be used as a therapeutic agent to inhibit immune responses in a fashion similar to that know for the HLA-B2702.75-84 peptide.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

A. Interaction of HLA-B2702.75-84 with HSP70

In the Examples, data are presented which demonstrates that the immunosuppressive activity of the HLA-B2702.75-84 peptide is potentiated, in part, by the interaction of HLA-B2702.75-84 with a member of the HSP70 family of

proteins. Upon interaction, immune responses, such as CTL mediated killing, are repressed. Based on these observations, one aspect of the present invention provides the specific interactions which mediate the immunorepressive activity of the HLA-B2702.75-84 peptide.

As described below, this interaction can be used: 1) to identify and isolate new immunomodulating agents, 2) in methods to identify agents which block the association of the HLA-B2702.75-84 peptide with HSP70, and 3) as a target to rationally design immunomodulating agents

B. Methods to identify agents which modulate immune activity

The present invention further provides methods for identifying agents which modulate immune activity by mimicking the HLA-B2702.75-84/HSP70 interaction. As provided above, the immunomodulating activity of the HLA-B2702.75-84 peptide has been shown to be based on the ability of the HLA-B2702.75-84 peptide to bind to a member of the HSP70 family of proteins. Knowledge of this interaction provides a basis of identifying new immunomodulating agents. Such new agents are said to mimic HLA-B2702.75-84/HSP70 interaction when the agent can reduce or block the association of the HLA-B2702.75-84 peptide with HSP70. Such agents can be selected as having an equivalent activity as the HLA-B2702.75-84 peptide, as having a more selective activity than the HLA-B2702.75-84 peptide, or as having a greater activity than the HLA-B2702.75-84 peptide.

Specifically, to identify a new agent, the HLA-B2702.75-84 peptide, a fragment thereof, or a protein containing the HLA-B2702.75-84 peptide (hereinafter collectively "the HLA-B2702.75-84 peptide"), is mixed with isolated HSP70, an isolated fragment of HSP70, or a cell capable of expressing HSP70 or a HSP70 fragment (hereinafter collectively "the HSP70"), in the presence and absence of an agent to be tested. After mixing under conditions which allow association of the HLA-B2702.75-84 peptide with the HSP70, the two mixtures are analyzed and compared to determine if the agent blocked or reduced the amount of binding of the HLA-B2702.75-84 peptide with the HSP70. Agents which block or decrease the binding of

the HLA-B2702.75-84 peptide with the HSP70 will be identified as decreasing the amount of binding present in the sample containing the tested agent.

As used herein, an agent is said to block or decrease HLA-B2702.75-84/HSP70 binding when the presence of the agent prevents or reduces the amount of association of the HSP70 with the HLA-B2702.75-84 peptide. One class of agents will reduce or block the association by binding to the HSP70 while another class of agents will reduce or block the association by binding to the HLA-B2702.75-84 peptide.

The HLA-B2702.75-84 peptide used in the present method can either be the entire HLA-B2702.75-84 peptide, with the amino acid sequence RENLRIALRY (Sequence ID NO: 1), a fragment of the HLA-B2702.75-84 peptide which binds the HSP70, or a protein which contains the HLA-B2702.75-84 amino acid sequence, such as an isolated HLA subunit, a fusion protein containing the HLA-B2702.75-84 sequence, or a larger HLA fragment such as HLA-B2702.60-84. In the examples that follow, a synthetic peptide corresponding to the HLA-B2702.75-84 peptide is used. Additionally, the HLA-B2702.75-84 containing peptide can contain more than one copy of the HLA-B2702.75-84 sequence, such as in a palidromic or tandem repeat.

As an alternative to compounds containing the HLA-B2702.75-84 peptide, agents identified in the present method can be substituted for the HLA-B2702.75-84 peptide. For example, an agent which is found to block HLA-B2702.75-84/HSP70 binding can be used in place of the HLA-B2702.75-84 peptide for further screening of compounds.

The HSP70 used in the present method can be any isolated member of the HSP70 family of proteins so long as the member binds the HLA-B2702.75-84 peptide. The HSP70 family member can be used in its entirety or a fragment of the HSP70 protein which contains the HLA-B2702.75-84 binding site can be used. Alternatively, a cell expressing the HSP70, or HSP70 fragment, can be used.

As used herein, an HSP70 family member refers to proteins currently known in the art which are members of the HSP70 family of proteins (for a review see The

Biology of Heat Shock Proteins and Molecular Chaperones 1994. Cold Spring Harbor Laboratory Press.) These include, but are not limited to HSP70, a peptide which is constitutively expressed In some human cells but whose expression is highly enhanced under exposure to high temperature or stress and the constitutively expressed protein, HSC70.

The HLA-B2702.75-84 and HSP70 peptides/proteins used in the present invention can be used in a variety of forms. The proteins can be used in a highly purified form, free of naturally occurring contaminants. Alternatively, a crude preparation containing a mixture of cellular components as well as the HLA-B2702.75-84 and HSP70 proteins can be used. So long as the association of the HSP70 with the agent to be tested and/or the HLA-B2702.75-84 peptide can be identified in the sample, the HLA-B2702.75-84 and HSP70 proteins are in a suitable form for use in the above described assay. Additionally, the HLA-B2702.75-84 and/or HSP70 proteins can be modified to contain a detectable label/signal generation system to facilitate detection. Methods for attaching agents such as florescence tags and secondary labeling agents such as biotin, are well known in the art.

A variety of art known methods can be adapted and employed to detect whether an agent blocks or reduces the interaction of the HLA-B2702.75-84 peptide with the HSP70. Such methods include, but are not limited to, assays which employ a solid support, assays in solution phase, assays performed in a gel-type media, and assays which use a combination of these environments. An example of a solid phase assay would be one in which one or more of the HLA-B2702.75-84 or the HSP70 peptides are immobilized on a solid support and is incubated in a solution phase with the agent to be tested and the other peptide of the HLA-B2702.75-84/HSP70 pair. A secondary detection means, such as an antibody is then used to determine the amount of the second peptide which binds to the immobilized peptide. Alternatively, the second peptide of the HLA-B2702.75-84/HSP70 pair can be detectably labeled and its binding to the immobilized first peptide directly assessed. One format which is preferably suitable for a solid phase based assay is immobilization of one of the

members in a 96-well micro-titer plate. Such titer plates provide an efficient assay format for rapidly processing multiple samples.

Alternatively, both peptides of the HLA-B2702.75-84/HSP70 binding pair can be in solution. After mixing, the binding of the HLA-B2702.75-84 peptide to the HSP70 can be detected using a variety of methods, for example detecting mobility shifts using electrophoretic means. One skilled in the art can readily appreciate how numerous assay-type formats which are known in the art for use in competitive assays can be modified to use the HLA-B2702.75-84/HSP70 pair.

Direct binding to the HLA-B2702.75-84 peptide of the HSP70 can be used as first step in identifying agents which block HLA-B2702.75-84/HSP70 interaction. For example, in such methods, agents are first screened for the ability to bind HSP70. Agents which bind HSP70 are then screened for the ability to block HLA-B2702.75-84/HSP70 interaction, or for the ability to modulate a function of the immune system.

Agents which are assayed in the above methods can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the association of the HLA-B2702.75-84 peptide with the HSP70. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library.

As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis which takes into account the sequence of the target site and/or its conformation in connection with the agent's action. As described above, two sites of actions for agents of the present invention are the HLA protein and the HSP70. Agents can be rationally selected or rationally designed by utilizing the peptide sequences which make up the contact sites of the HLA-B2702.75-84/HSP70 pair. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to the HLA-B2702.75-84 contact site found on the HSP70.

Such an agent will reduce or block the association of the HLA-B2702.75-84 peptide with the HSP70 by binding to the HLA.

The agents of the present invention can be peptides, including peptides containing modified amino acids, vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention or those used in the present assay methods.

One class of agents of the present invention are peptide agents whose amino acid sequences is chosen based on the amino acid sequence of the HLA-B2702.75-84 peptide or the HLA-B2702.75-84 contact site found on the HSP70. The HLA-B2702.75-84 contact site on HSP70 can readily be determined using art-known methodologies. For example, tryptic digestion of the HSP70 protein can be performed and the various fragments of the HSP70 can be tested for their ability to bind the HLA-B2702.75-84 peptide. Alternatively, a modification of a bind and chew assay can be used in which the HLA-B2702.75-84 and HSP70 peptides are allowed to interact and the interactive pair is subject to protein digestion. Regions of the HSP70 which are contacted by the HLA-B2702.75-84 peptide will be protected from digestion and can be later characterized to determine the amino acid sequence which is bound and protected.

All of the peptide agents of the invention, when an amino acid forms the C-terminus, may be in the form of the pharmaceutically acceptable salts or esters. Salts may be, for example, Na^+, K^+, Ca^{+2}, Mg^{+2} and the like; the esters are generally those of alcohols of 1-6C. In all of the peptides of the invention, one or more amide linkages (-CO-NH-) may optionally be replaced with another linkage which is an isostere such as $-CH_2NH-$, $-CH_2S-$, $-CH_2CH_2-$, -CH=CH- (cis and trans), $-COCH_2-$, $-CH(OH)CH_2-$ and $-CH_2SO-$. This replacement can be made by methods known in the art. Alternative peptide linking moieties can also be used to decrease the rate of degradation of peptide based agents. The following references describe preparation of peptide analogs which include these alternative-linking moieties: Spatola, A.F., Vega Data (March 1983), Vol. 1, Issue 3, "Peptide Backbone Modifications" (general review); Spatola, A.F., in "Chemistry and Biochemistry of Amino Acids Peptides and

Proteins," B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983) (general review); Spatola, A.F., *et al., Life Sci.* 38:1243-1249 (1986)(-CH_2-S); Hann, M.M., *J Chem Soc Perkin Trans I* 307-314 (1982) (-CH-CH-, cis and trans); Almquist, R.G., *et al., J Med Chem* 23:1392-1398 (1980) (-$COCH_2$-); Jennings-White, C., *et al., Tetrahedron Lett* 23:2533 (1982)(-$COCH_2$-); Holladay, M.W., *et al., Tetrahedron Lett* 24:4401-4404 (1983)(-C(OH)CH_2-); and Hruby, V.J., *Life Sci* 31:189-199 (1982)(-CH_2-S-).

Antipeptide peptides are another type of rationally designed peptide agent of the present invention. Antipeptide agents are peptides whose amino-acid sequence is specifically selected so as to interact with a target peptide sequence. Antipeptides can be designed using art-known methods, for example, see T.K. Gartner *et al., Biochem. Biophys. Res. Commun.* 180:1446-1452, (1991).

Another class of agents of the present invention are antibodies immunoreactive with critical positions of the HLA-B2702.75-84 peptide or with the HSP70. Antibody agents are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the HLA-B2702.75-84 peptide or HSP70 which are intended to be targeted by the antibodies. Critical regions include, but are not limited to, the contact sites involved in the association of the HLA-B2702.75-84 with the HSP70 and sights which provide steric interference upon binding.

Antibody agents are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using the peptide haptens alone, if they are of sufficient length, or, if desired, or if required to enhance immunogenicity, conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be desirable to provide accessibility to the hapten. The hapten peptides can be extended at the amino or carboxy terminus with a Cys residue or interspersed with cysteine residues, for example, to facilitate linking to carrier.

Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers of antibodies are taken to determine adequacy of antibody formation.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations is preferred. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler and Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. (See Harlow: Antibodies Cold Spring Harbor Press NY 1989) The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten or is the HSP70 or HLA-B2702.74-85 peptide. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either in vitro or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab', of $F(ab')_2$ fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of receptor can also be produced in the context of chimeras with multiple species origin.

The antibodies thus produced are useful not only modulators of immune function, but are also useful in immunoassays.

C. Uses for agents which block the association of HLA-B2702.75-84 with a member of the HSP70 family of proteins

As provided in the Background section, the HLA-B2702.75-84 peptide has been shown to modulate a variety of biological responses, particularly those involving the immune system. In particular, the HLA-B2702.75-84 peptide has been shown to be a potent inhibitor of CTL mediated killing and has found use in increasing allograft tolerance and reducing the severity of autoimmune disorders such as rheumatoid arthritis. Further, agents which bind to members of the HSP70 family have been shown to modulate overlapping biological responses, and in particular the immunosuppressive compound, deoxyspergualin, has been shown to bind HSP70 (Nadler *Science* 258:484-486 (1992)). Therefore, agents which block or reduce HLA-B2702.75-84/HSP70 binding can be used to modulate immune system responsiveness in the same fashion as shown for the HLA-B2702.75-84 peptide and immunomodulatory agents which bind HSP70.

Specifically, immune system activity, such as T-cell mediated responses, can be modulated by administering to a subject an agent which can block the interaction of the HLA-B2702.75-84 peptide with a member of the HSP70 family. As used herein, a subject can be any mammal, so long as the mammal is in need of modulation of immune activity. The term "mammal" is meant an individual belonging to the class Mammalia. The invention is particularly useful in the treatment of human subjects.

As used herein, immune system activity refers to the wide variety of cellular events in which cells of the immune system participate. In general, the HLA-B2702.75-84 peptide has been shown to selectively inhibit events mediated by T-cells and T-cell activation, and in particular CTL mediated killing. Examples of situations where it is desirable to modulated such activity includes, but are not limited to, transplant surgery and autoimmune disorders. In each of these situations, it is desirable to selectively reduce T-cell responsiveness.

As used herein, an agent is said to modulate a immune system activity, or reduce the severity of a pathological condition mediated by the immune system, when

the agent prevents the normal immune activity of the subject. For example, an agent is said to modulate graft rejection when the agent reduces the rate of onset of graft rejection or reduces the severity of graft rejection.

D. Administration of Agents which effect modulate immune system activity

The agents of the present invention can be provided alone, or in combination with another agent that modulates a function of the immune system. For example, an agent of the present invention used to that reduce graft rejection can be administered in combination with other immunosuppressive agents. As used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that the agents will act at the same time.

The agents of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The present invention further provides compositions containing one or more agents of the present invention. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages comprise 0.1 to 100 mg/kg/body wt. The preferred dosages comprise 1 to 100 mg/kg/body wt. The most preferred dosages comprise 10 to 100 mg/kg/body wt.

In addition to the pharmacologically active agent, a composition comprising an agent of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble

salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulation may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

E. Methods for targeting the pharmaceutical agents of the present invention

The present invention further provides methods for increasing the affinity of the agents of the present invention, as well as other known agents which block or reduce HLA-B2702.75-84/HSP70 interaction. Specifically the affinity of an agent which blocks the HLA-B2702.75-84/HSP70 interaction can be increased by covalently linking the agent to a second agent which has a equal or higher affinity for either HSP70 or HLA. Such a second agent will bind to another site on either the HLA or HSP70 molecule and bring the HLA-B2702.75-84/HSP70 blocking agent into close proximity to the target site. Such second agents can be, but are not limited to, antibody and peptide agents. The second agent can be covalently attached to the HLA-B2702.75-84/HSP70 blocking agent using art know methods. Methods which employ linkers are particularly well suited for this use.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and

utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLE I

Isolation and Characterization of the HLA-B2702.75-84 Binding Partner

A CTL line was labeled with ^{35}S-methionine and cysteine. The cells were then divided into two groups. One group was incubated at 30°C for 60 min with the following peptides:

Biotin-(CH)12-YRLAIRLNERRENLRIALRY (The B2702 palindrome dimer) (Seq. ID No:2)

Biotin (CH)12-YGRLNRLSERRESLRNLRGY) (The B7 palindrome dimer)(Seq. ID No:3)

The cell/peptide complexes were washed extensively and solubilized with CHAPS. The material was then incubated with streptavidin conjugated to agarose beads.

The other group of cells was first lysed in CHAPS and then incubated with the biotin-peptide-streptavidin complex. All samples were washed extensively, boiled in SDS, and separated by SDS-PAGE and subjected to fluorography. A prominent band of approximately 75-80kD was detected in the lanes with the B2702 peptide, but not the B7 peptide, nor the streptavidin alone lanes, for both methods of preparation. The same band was observed under the same conditions with activated CTL, the cell tumor PEER, and the EBV transformed cell line JY.

To further elucidate the mechanism by which the B2702 peptide mediates T cells, experiments were designed to identify peptide binding proteins. In the following Example, evidence is presented which demonstrates that the inhibitory peptide B2702 binds two proteins with apparent molecular masses of 70kD and 74kD which are

members of the heat-shock protein (HSP) 70 family. The binding is sequence specific and restricted to peptides with T cell inhibitory activity. The physiologic significance of this binding and the potential role of HSP70 family members in mediating the T cell immunomodulatory effects is discussed. Based on these observations, the present invention provides methods to identify agents which modulate T cell activity by blocking the interaction of HSP 70 with a member of the HLA family of proteins.

EXAMPLE II

Materials and Methods

HLA-derived Peptides. All peptides were synthesized as described in Clayberger, C., *et al. Transplantation Proc.* (1993) 25:477-478. The peptides synthesized include (Table 1): peptide B0701.75- 84, peptide B2702.75-84 and peptide B2705.75-84 corresponding to amino acids 75-84 of the alpha 1 helix of the HLA-B0701, HLA-B2702 and HLA-B2705 molecules. Amino acid residues which comprise the "public epitope" Bw4a (B2702) or Bw6a (B0701), respectively, are underlined in Table 1. HLA class I heavy chain sequences are from Zemmour and Parham (Zemmour, J., *et al. Immunogenetics* (1993) 37:239-250). B2702.84-75/75-84 and B2702.84-75/75-84 represent inverted repeat dimers of amino acids 75-84 of the respective HLA class I molecule and peptide B2702.75-84/75-84 is a direct repeat of amino acids 75-84. B2702.84-75T/75-84, B2702.84-75/75-84T and B2702.84-75T/75-84T contain single or double threonine substitutions (see Table 1).

For biochemical analyses, a biotin group was attached to the N-terminal amino acid using N-hydroxy-succinimidyl-ester activated (NHS) biotin (NHS-LC-Biotin II, Pierce Chemical Co., Rockford, IL). Attachment of the biotin group had no effect on the T cell immunomodulatory activities of the peptides. Direct and inverted repeat dimeric peptides performed similarly in T cell assays as well as in biochemical analyses with slightly enhanced activities over the monomeric versions. The studies presented in this Example were performed with the inverted repeat dimeric peptides B2702.84-75/75-84 and B0701.84-75/75-84, abbreviated as 02/02 and B7/B7.

Cells and Antibodies. Peripheral blood lymphocytes (PBL) were isolated from venous blood of healthy donors via Hypaque-Ficoll density centrifugation. Cytotoxic T cell lines (CTL) were established from PBL by stimulation with irradiated B-lymphoblastoid cell lines (B-LCL) (allogeneic stimulation) (Buxton, S.E., *et al. J. Exp. Med.* (1992) 175:809-820). Long-term CTL cultures were carried in T cell conditioned medium (Krensky, A.M., *Proc. Natl. Acad. Sci. USA* (1982) 79:2365-2369) by weekly stimulation with irradiated B-LCL. Antibodies specific for various stress proteins were purchased from StressGen (Victoria, BC, Canada): 1B5 (rat IgG1a) recognizes the constitutively-expressed (HSC70) member of the HSP70 family, C92 (mouse IgG1) is specific for the heat-inducible (HSP70) member of the HSP70 family. 10C3 (mouse IgG2a) recognizes the glucose-regulated proteins grp78/BiP and grp94. The anti-grp75 rabbit-polyclonal antiserum (specific for the mitochondrial glucose-regulated protein, grp75) was a gift from Dr. W. Welch (San Francisco, CA). Anti-Hsp72 is a mouse ascites (RPN1197, IgG1) (Amersham, Arlington Heights, IL) with identical specificity to C92. Isotype matched antibodies (Caltag Laboratories, South San Francisco, CA) were used as controls in immune precipitation assays.

Heat-shock Treatment. Cells were incubated at 43°C for 1 h and allowed to recover for 3 h at 37°C before metabolic labeling or precipitations were performed.

Metabolic Radiolabeling. For radiolabeling, CTL were used on day 4 or day 5 after allogeneic stimulation. 3 x 10^6 cells/ml were pre-incubated in methionine/cysteine free RPMI 1640 (ICN Biomedical Inc., Costa Mesa, CA) supplemented with 5 % dialyzed FCS, 2 mM L-glutamine and 1.4 mM sodium pyruvate for 1 h. Then [^{35}S] methionine/cysteine (50 µCi/ml of ProMix, Amersham) was added to the culture and the cells further incubated for 4 h. After radiolabeling, cells were washed twice in ice-cold PBS.

Preparation of Peptide Affinity-matrix. Saturating amounts of biotinylated peptide were incubated with Streptavidin-agarose beads (Pierce) for 2 h at room temperature. Before use in precipitation assays, unbound peptide was removed by extensive washing in PBS.

Precipitation of Peptide Binding Proteins. For each precipitation, 5-7 x 10^6 radiolabeled CTL were used. Cell pellets were lysed in 500 µl of 0.6 % CHAPS/PBS pH 7.4 containing protease inhibitors, pepstatin (1 µg/ml), leupeptin (10 µg/ml) and PMSF (0.2 mM) on ice for 30 min. Cell debris was spun out by microcentrifugation for 10 min at 4°C. Peptide binding proteins were isolated by incubating cell lysates with peptide/Streptavidin-agarose beads for 1.5 h at 4°C. Where indicated, 5 mM of either ATP or gS-ATP (Boehringer Mannheim, Indianapolis, IN) were added simultaneously with the peptide matrix. After incubation, Streptavidin-agarose beads were pelleted from the lysate and washed sequentially in one ml of each: 0.6 % of CHAPS/PBS; TNEN (0.5 % NP40, 20 mM Tris HCl pH 7.6, 0.1 M NaCl, 10 mM EDTA) diluted 1/10 in dH_2O and supplemented with 0.05 % deoxycholic acid, 0.01 % SDS; and a 1/10 dilution of TNEN containing 0.5 M NaCl.

Precipitation of Stress Proteins. Radiolabeled cell lysates were incubated with 5 µl of normal rabbit serum and 250 µl of a 10 % solution of protein A positive *S. aureus* cells (Boehringer Mannheim) for 2-4 h at 4°C (pre-clearing). *S. aureus* cell were spun out and the precleared lysates immunoprecipitated with 5 µg of purified monoclonal antibody or 10 µl of rabbit antiserum or ascites fluid and 50 µl of packed ProteinG-Sepharose beads (Pharmacia Biotech Inc., Alameda, CA) for 1.5 h at 4°C. Immune complexes were collected by centrifugation and washed sequentially in one ml of each: TNEN supplemented with SDS (0.1 %) and deoxycholic acid (0.5 %); TNEN, diluted 1/10 in dH_2O and supplemented with 0.5 M NaCl.

Electrophoresis. Precipitated proteins were separated by reducing SDS-PAGE (Lämmli, U.K. *Nature* (1970) 227:680-685) or isoelectric focusing electrophoresis (IEF) (Yang, S.Y. (1987) 332-335).

Western Blot Analysis. Peptide binding proteins were precipitated from unlabeled cell lysates as described above. After separation on SDS-PAGE, proteins were transferred to PVDF membrane (Millipore Corporation, Bedford, MA). Immunodetection using enhanced chemiluminescence (ECL, Amersham) was performed following the manufacturer's instruction. The membrane was probed first

with anti-HSP70 antibody (C92, 1:2000) and horseradish peroxidase conjugated anti-mouse antibody (1:5000). After ECL-detection, the membrane was stripped off bound antibodies by incubating in a solution of 100 mM ß-mercaptoethanol/2 % SDS/62.5 mM Tris-HCl pH 6.8 for 30 min at 50°C. Then the membrane was re-probed using anti-HSC70 antibody (1B5, 1:5000) and horseradish peroxidase conjugated anti-rat antibody (1:5000).

One-dimensional Peptide Mapping using V8 Endoproteinase Digestion. Radiolabeled protein bands were eluted from non-fixed, dried SDS-PAGE gels in PBS/1 % SDS over two days. After acetone precipitation, proteins were solubilized in 40 µl of V8 digestion buffer (0.125 mM Tris-HCl pH 6.8, containing 10 % glycerol, 1 mM EDTA, 0.1 % SDS and 0.001 % bromphenol blue) and loaded onto 15 % SDS-PAGE. Digestion was performed "in gel" (Cleveland, D.W., *et al. J. Biol. Chem.* (1977) 252:1102-1106; Fischer, S.G. *Meth. Enzymol.* (1983) 100:424-430) by overlaying the protein solution with 0.5 µg of *S. aureus* V8 endoproteinase (Boehringer Mannheim) and running the sample through the stacking gel slowly. At the stacking/separating gel interface, the gel run was interrupted for 30 min to allow further digestion. Then, electrophoresis was performed as usual (Lämmli, U.K. *Nature* (1970) 227:680-685).

Cell-mediated Cytotoxicity Assay. Cell killing was measured using a standard 4 h ^{51}chromium-radioisotope release assay (Krensky, A.M., *Proc. Natl. Acad. Sci. USA* (1982) 79:2365-2369). Briefly, CTL lines specific for allogeneic HLA-class I molecules were cultured as described above. B-LCLs expressing the HLA-class I molecule recognized by the CTL line were labeled with ^{51}chromium (Amersham) for 1 h and added to the CTL at an effector to target cell ratio such that the percent of specific lysis in the untreated samples was between 30 % and 50 %. HLA-derived peptides (0 µg/ml to 50 µg/ml) were added to the CTL simultaneously with the target cells and remained present during the 4 h incubation. All assays were performed in triplicate. The percent of specific lysis was calculated by the formula: [(experimental cpm - spontaneous cpm)/(total cpm - spontaneous cpm)] x 100. Non-biotinylated and biotinylated peptides gave identical results.

Results and Discussion

The T cell inhibitory peptide B2702 binds to proteins with apparent molecular masses of 74kD and 70kD. Synthetic peptides corresponding to amino acid sequences of the alpha 1 helix of HLA class I molecules which overlap the "public epitope" Bw4/Bw6 were found to inhibit T lymphocyte function in vitro (Clayberger, C., *et al. J. Immunol.* (1990) 144:4172-4176; Clayberger, C., *et al. Transplantation Proc.* (1993) 25:477-478; Krensky, A.M., *et al. Curr. Opin. Immunol.* (1994) 6:791-796) and to result in allograft tolerance in transplantation models (Nisco, S., *et al. J. Immunol.* (1994) 152:3786-3792; Buelow, R., *et al. Transplantation* (1995) 59:455-460; Cuturi, M.-C., *et al. Transplantation* (1995) 59:661-669). The peptide effects were not allele-specific. Therefore, these HLA-derived peptides are of particular interest in transplant therapy, as they can potentially be used for all donor-recipient combinations. Peptides with sequences corresponding to the Bw4a epitope had most potent effects in that they inhibited the differentiation of T cell precursors into mature CTL and also inhibited target cell lysis by mature CTL (Clayberger, C., *et al. Transplantation Proc.* (1993) 25:477-478; Krensky, A.M., *et al. Curr. Opin. Immunol.* (1994) 6:791-796).

To elucidate the mechanism through which these HLA-derived peptides mediate their T cell-inhibitory effect, peptide binding proteins were isolated from radiolabeled T cell lysates using Streptavidin-agarose beads conjugated with biotinylated peptides. Precipitations were performed with the inverted repeat dimeric peptides, B2702.84-75/75-84 and B0701.84-75/75-84, in parallel. Two proteins with molecular masses of 70kD and 74kD were identified which bound to the inhibitory peptide B2702, but not to the non-inhibitory peptide B0701. Additional bands of 78kD and 50kD were found with both the B2702 and B7 peptide and also with Streptavidin-agarose in the absence of any peptide, indicating that their binding is not peptide dependent. When the lysis buffer was supplemented with ATP, the B2702 peptide no longer bound to the 74kD and 70kD proteins. The non-hydrolysable ATP-

derivative, gS-ATP, had no effect, suggesting that ATP hydrolysis abrogates the binding of the B2702 peptide to the 74kD and 70kD proteins.

Heat-shock treatment of cells before metabolic labeling resulted in a dramatic increase in intensity of the 70kD band and a modest increase for the 74kD band. The weak signal observed for the 70kD protein under normal growth conditions, therefore, reflects lower quantities of the radiolabeled 70kD protein present in cells grown under normal conditions, rather than lower affinity of the B2702 peptide for the 70kD protein compared to the 74kD protein.

The 74kD and 70kD peptide binding proteins correspond to the constitutively expressed (HSC70) and heat-inducible (HSP70) members of the HSP70 family. Molecular mass, regulation of binding by ATP, and the upregulation upon heat-stress suggested that the B2702 peptide binding proteins are members of the heat-shock protein 70 (HSP70) family. Different members of the HSP70 family were precipitated with respective antibodies and compared to the B2702 peptide binding proteins on SDS-PAGE and IEF. Two glucose-regulated family members, grp78 and grp75, were tested and found to differ in molecular mass and isoelectric point from the 74kD and 70kD peptide binding proteins. HSC70 and HSP70 are two highly related family members which differ in their expression pattern. HSC70 is expressed constitutively (therefore called HSC70) in all cells and only moderately upregulated upon heat-shock. HSP70, also called the inducible family member, is expressed constitutively only in some human cells, but its expression is highly enhanced upon exposure to high temperature. The 74kD and 70kD peptide binding proteins closely resemble the HSC70 and HSP70 proteins in electrophoretic mobility on SDS-PAGE and IEF and display identical response to heat. Furthermore, in Western blot analysis the 74kD peptide binding protein was recognized by 1B5 (specific for HSC70) and the 70kD peptide binding protein was recognized by C92 (specific for HSP70). Thus, the 74kD and 70kD peptide binding proteins appeared to correspond to HSC70 and HSP70 proteins, respectively. This hypothesis was confirmed by digestion with V8 endoproteinase, which created identical patterns for the 74kD protein and HSC70 and the 70kD protein and HSP70.

Peptide binding to HSC/HSP70 is sequence-specific and correlates with the peptide effects on T lymphocyte function. HSP70 family members are known to bind peptides and the binding motifs have been described recently (Fourie, A.M., *et al. J. Biol. Chem.* (1994) 269:30470-30478). Alternating hydrophobic or aromatic residues with relative positions 2, 4, and 6 (P2, P4, P6) in the peptide sequence were defined as a motif. In addition, HSC70 favors the binding of peptides with positively charged residues. The B2702 peptide sequence, RENLRIALRY, fulfills these binding requirements precisely. The amino acid residues underlined (L, I, L) correspond to the defined hydrophobic anchors, P2, P4, and P6, of the binding motif. The B0701 peptide sequence, RESLNRLRGY, does not match the binding motif. Furthermore, Gething *et al.* (Fourie, A.M., *et al. J. Biol. Chem.* (1994) 269:30470-30478) showed that amino acid substitutions at position 4 (P4) in the peptide sequence in particular resulted in significantly reduced binding affinity, indicating position 4 as the main anchor position. Aligning the B2702 sequence to fit the binding motif, the isoleucine residue corresponds to position P4 and is likely to be the main anchor residue for the B2702 peptide. This hypothesis was confirmed by binding studies. Exchanging the first isoleucines for threonine in the B2702.84-75/75-84 inverted repeat peptide resulted in slightly reduced binding to HSC/HSP70. Significantly reduced binding was observed when the second isoleucine was replaced for threonine and exchanging both isoleucines resulted in a peptide, B2702T/T, which no longer bound to HSC70 or HSP70. Thus the binding motif provides an explanation for the failure of the B0701 and B2702T/T peptides to precipitate the 74kD and 70kD molecules.

Most interesting, however, is that the importance of isoleucine in the peptide sequence had previously been defined functionally by comparing peptides derived from closely related HLA-B27 allotype sequences (Clayberger, C., *et al. Transplantation Proc.* (1993) 25:477-478). HLA-B2702 and HLA-B2705 are closely related proteins which differ by only three amino acids in the alpha 1 helix (Table 1). Synthetic peptides corresponding to that region were investigated in *in vitro* T cell assays and the B2705 peptide was found ineffective while the B2702 peptide inhibited T cell function (Clayberger, C., *et al. Transplantation Proc.* (1993) 25:477-478). When the

B2705 amino acid residues were singly introduced into the B2702 peptide sequence, only the substitution of isoleucine for threonine at position 80 caused a loss of the functional effects. The effect of substituting the isoleucine residue was further analyzed using the inverted repeat peptide, B2702.84-75/75-84. The inhibitory effect was reduced when one or the other of the isoleucines was replaced for threonine and exchanging both the isoleucines resulted in a complete loss of function. This loss of function correlated strictly with the loss of binding to the HSC/HSP70 proteins (Table 2).

The binding of B2702 peptides and not B0701 or B2702T/T peptides to HSC/HSP70 proteins can be explained on the basis of the defined HSC70 binding motif. Most intriguing, however, is the correlation between the functional effects and the binding to HSC/HSP70. Without exception to date, peptides with T cell inhibitory effects bind to HSC/HSP70, whereas non-inhibitory peptides do not bind. This correlation suggests that the binding to HSC/HSP70 might be of physiological relevance and involved in mediating the T cell immunomodulatory effect of the HLA-derived B2702 peptide.

Heat-shock proteins as modulators of the immune response. The analysis of immunosuppressive compounds like cyclosporin A (CsA) and FK506 has substantially increased our understanding of signaling pathways involved in T lymphocyte activation (Fourie, A.M., *et al. J. Biol. Chem.* (1994) 269:30470-30478). Two classes of immunosuppressant binding proteins, collectively called immunophilins, have been identified and the binding of the immunosuppressant to its immunophilin is required to mediate the immunosuppressive effect. The immunophilins of both CsA and FK506 are abundantly expressed proteins which are conserved throughout evolution. They are found in different isoforms and are localized to various subcellular compartments. In addition, both exhibit rotamase activity and are postulated to be involved in protein folding, reduction of protein aggregates and protein translocation (Fruman, D.A., *et al. FASEB J.* (1994) 8:391-400). These characteristics also apply to HSP70 proteins (Gething, M-J., *et al. Nature* (1992) 355:33-45). Of interest, it has been reported that the immunosuppressive drug, deoxyspergualin (DSG), binds to HSP70 family members

(Nadler, S.G., *et al. Science* (1992) 258:484-486). Based upon the biochemical similarities between immunophilins and HSP70 proteins and their ability to bind immunosuppressive compounds, it has been suggested that heat-shock protein 70 family members might represent a third class of immunophilins (Nadler, S.G., *et al. Science* (1992) 258:484-486). This hypothesis is now further substantiated by our finding that T cell inhibitory HLA-derived peptides bind to HSC70 and HSP70.

The role of heat-shock proteins in antigen presentation and tumor immunity is currently an area of intense investigation (Vanbuskirk, A., *et al. J. Exp. Med.* (1989) 170:1799-1809; Manara, G.C., *et al. Blood* (1993) 82:2865-2871; Udono, H., *et al. J. Exp. Med.* (1993) 178:1391-1396; Hightower, L.E., *et al.* (1994) 179-207; Srivastava, P.K., *et al. Immunogenetics* (1994) 39:93-98). Recently, it has been demonstrated that DSG exerts its immunosuppressive activity at the level of the monocytes, apparently by interfering with their antigen presentation function (Hoeger, P.H., *et al. J. Immunol.* (1994) 153:3908-3916). Therefore, the DSG system provides substantiating evidence for the hypothesis previously proposed by Srivastava and co-workers (Srivastava, P.K., *et al. Immunogenetics* (1994) 39:93-98) implicating HSP70 in antigen presentation pathways.

DSG and the HLA-derived B2702 peptide, although apparently binding to the same ligand HSC70, differ in their immunomodulatory activity. First, the HLA-derived peptide inhibits allospecific cytotoxic T cells and inhibits T cell proliferation, while DSG does not inhibit cytolysis. Second, the HLA-derived peptide effects are exerted at the level of the T cell, not at the level of the antigen-presenting cell as demonstrated for DSG (Hoeger, P.H., *et al. J. Immunol.* (1994) 153:3908-3916). These differences suggest that the HLA-derived peptide mediates its immunosuppressive activities through a mechanism different than that described for DSG and that the role of HSC/HSP70 in the peptide's immunomodulation does not involve antigen presentation.

Consistent with the broad expression of HSC/HSP70 proteins, the 74kD/70kD peptide binding proteins were found in whole cell lysates of all cell types studied, including B-LCL, T cell tumor lines, HeLa, a pre-erythroid cell line (K562), and an endothelial cell line (SK-HEP1). The T cell specific peptide effect must, therefore, be

due to an additional mechanism not found in other cell types. Indicative of such a T cell specific mechanism is the observation that HSC/HSP70 proteins were isolated from T lymphocytes and CTL, only, when intact cells were incubated with B2702 peptides.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for identifying immunomodulating compounds, said method comprising the steps of:

a) mixing i) HSP70, a cell expressing HSP70, a fragment of HSP70 containing the HLA-B2702.75-84 binding site, or a cell expressing a fragment of HSP70 containing the HLA-B2702.75-84 binding site, and ii) a peptide which contains the HLA-B2702.75-84 peptide, or a cell expressing a peptide containing the HLA-B2702.75-84 sequence in the presence and absence of a candidate compound;

b) determining whether the presence of the candidate compound blocks or reduced the binding of the HSP70 to the HLA-B2702.75-84 peptide;

c) identifying immunomodulating compounds which blocks or reduces the binding of the HLA-B2702.75-84 peptide to HSP70.

2. The method of claim 1, wherein said peptide which contains the HLA-B2702.75-84 peptide is an isolated peptide whose amino acid sequence is depicted in Sequence ID.NO:1.

3. The method of claim 1, wherein said peptide which contains the HLA-B2702.75-84 peptide is an isolated peptide whose amino acid sequence is depicted in Sequence ID.NO:2.

4. The method of claim 1, wherein said peptide which contains the HLA-B2702.75-84 peptide is the HLA-B2702.60-84 peptide.

5. The method of claim 1, wherein said HSP70 is human HSP70.

6. The method of claim 1, wherein said HSP70 is human HSC70.

7. The method of claim 1 wherein said HSP70 is a fragment of a protein of the HSP70 family of proteins which binds to the HLA-B2702.75-84 peptide.

8. The method of claim 1, wherein one or both of said peptides which contains the HLA-B2702.75-84 peptide or the HLA-B2702.75-84 peptide binding site of HSP70 is expressed on the surface of a cell.

9. The method of claim 1, wherein one or both of said peptides which contains the HLA-B2702.75-84 peptide or the HLA-B2702.75-84 peptide binding site of HSP70 is provided as a fusion protein.

10. The method of claim 1, wherein one or both of said peptides which contains the HLA-B2702.75-84 or the HLA-B2702.75-84 peptide binding site of HSP70 is detectably labeled.

11. The method of claim 1, wherein one or both of said peptides which contains the HLA-B2702.75-84 or the HLA-B2702.75-84 peptide binding site of HSP70 is immobilized on a solid support.

12. The method of claim 1, wherein one or both of said peptides which contains the HLA-B2702.75-84 or the HLA-B2702.75-84 peptide binding site of HSP70 is detectably labeled.

13. The method of claim 1 wherein said agent is first tested for the ability to bind to a member of the HSP70 family of proteins.

14. An isolated agent which blocks the binding of the HLA-B2702.75-84 peptide to HSP70.

15. An isolated agent which blocks the binding of the HLA-B2702.75-84 peptide to HSP70, wherein said agent is identified by the steps of:

a) mixing i) HSP70, a cell expressing HSP70, a fragment of HSP70 containing the HLA-B2702.75-84 binding site, or a cell expressing a fragment of HSP70 containing the HLA-B2702.75-84 binding site, and ii) a peptide which contains the HLA-B2702.75-84 peptide, or a cell expressing a peptide containing the HLA-B2702.75-84 peptide in the presence and absence of a candidate compound;

b) determining whether the presence of the candidate compound blocks or reduced the binding of the HSP70 to the HLA-B2702.75-84 peptide;

c) identifying immunomodulating compounds which blocks or reduces the binding of the HLA-B2702.75-84 peptide to HSP70.

16. A method to modulate an activity of the immune system in a subject, said method comprising the step of administering an effective amount of an agent which blocks the binding of the HLA-B2702.75-84 peptide to HSP70 to a subject whose immune system is to be modulated.

17. The method of claim 16 wherein said agent is identified by the steps of;

a) mixing i) HSP70, a cell expressing HSP70, a fragment of HSP70 containing the HLA-B2702.75-84 binding site, or a cell expressing a fragment of HSP70 containing the HLA-B2702.75-84 binding site, and ii) a peptide which contains the HLA-B2702.75-84 peptide, or a cell expressing a peptide containing the HLA-B2702.75-84 peptide in the presence and absence of a candidate compound;

b) determining whether the presence of the candidate compound blocks or reduced the binding of the HSP70 to the HLA-B2702.75-84 peptide;

c) identifying immunomodulating compounds which blocks or reduces the binding of the HLA-B2702.75-84 peptide to HSP70.

18. The method of claim 16, wherein said agent is administered to reduce T-cell activity.

19. The method of claim 16, wherein said agent is administered to reduce the rate of graft rejection.

20. The method of claim 16, wherein said agent is administered to reduce the severity of an autoimmune disorder.

21. The method of claim 20, wherein said agent is administered to reduce the severity of rheumatoid arthritis.

22. An isolated antibody which binds to HSP70, wherein said antibody blocks the binding of the HLA-B2702.75-84 peptide to said HSP70.

23. The antibody of claim 22, wherein said antibody binds to the HLA-B2702.75-84 peptide binding site on HSP70.

24. A pharmaceutical composition comprising an agent of claim 14 and a pharmaceutically acceptable excipient.

25. A pharmaceutical composition comprising an antibody of claim 22 and a pharmaceutically acceptable excipient.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/00117

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/00, 39/02; C07K 14/74, 14/195, 14/435; G01N 33/53
US CL :424/185.1, 193.1, 234.1; 435/7.1; 530/350, 395
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/185.1, 193.1, 234.1; 435/7.1; 530/350, 395

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Automated Patent System (APS) and DIALOG (file = biochem) databases: key words: heat shock proteins, HSP70, major histocompat? MHC? HLA?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 93/17699 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 16 September 1993, see entire document.	1-25
Y	CLAYBERGER et al. HLA-A2 peptides can regulate cytolysis by human allogeneic T lymphocytes. Nature. December 1987, Vol. 330, pages 763-765, see entire article.	1-25
Y	KRENSKY et al. The induction of tolerance to alloantigens using HLA-based synthetic peptides. Curr. Opin. Immunol. 1994, Vol. 6, pages 791-796, see entire article.	1-25
Y	CLAYBERGER et al. Peptides corresponding to T-cell receptor - HLA contact regions inhibit class I-restricted immune responses. Trans. Proc. 1993, Vol. 25, No. 1, pages 477-478, see entire article.	1-25

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
16 APRIL 1997	30 APR 1997

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	THOMAS M. CUNNINGHAM
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*